

## ABSTRACT

The present invention relates to a method for determining a nucleotide sequence of a nucleic acid by single dye molecule detection, the method comprising:

- (a) immobilizing a nucleic acid molecule, or a primer which has a  
5 sequence complementary to a part of the sequence of the nucleic acid molecule, onto the surface of a solid;
- (b) annealing the primer or the nucleic acid molecule to the nucleic acid molecule or the primer, respectively;
- (c) providing a solution which contains DNA polymerase and one type of  
10 dye-labeled dNTP, or RNA polymerase and one type of dye-labeled NTP, to the immobilized nucleic acid molecule, and allowing the nucleotide to react with the 3' end of the primer, whereby a nucleotide, which forms a base-pair with a base opposed to the reaction site, is bound to the primer by action of the polymerase;
- 15 (d) detecting the presence of a bound, dye-labeled dNTP or NTP;
- (e) disrupting the dye molecule of the bound, dye-labeled dNTP or NTP;
- (f) repeating the steps (3) to (5) while changing the type of dye-labeled dNTP or NTP in turn, to sequentially bind dNTPs or NTPs which base-pair with the nucleotides of the nucleic acid molecule; and
- 20 (g) determining the nucleotide sequence of the nucleic acid molecule based on the types of the sequentially bound dNTPs or NTPs.